the Reference Attenuated Poliovirus shall be considered acceptable and added to the previous testing experience only if the magnitude of its poliovirus neuropathology is statistically compatible with the results of all previous tests with the same reference preparations of the same type performed by the testing laboratory.

(5) *Alternative procedures in case of monkey shortage.* In the event of a shortage of test monkeys and upon approval of the Director, Center for Biologics Evaluation and Research, a monovalent virus pool may be tested without concurrent testing of the corresponding type Reference Attenuated Poliovirus. In such a case, the magnitude of the neuropathology of the monovalent virus pool shall be compared with the magnitude of the neuropathology exhibited in all previous tests of the corresponding Reference Attenuated Poliovirus.

§ 630.17 Alternative test for neurovirulence.

(a) In lieu of the neurovirulence test in § 630.16, the following test may be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot).

(b) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the Reference Attenuated Poliovirus, Type 1, for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ (mean tissue culture infectious dose) of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) *Intrathalamic inoculation.* Each of at least 30 monkeys shall be injected intracerebrally by placing 0.5 milliliter of virus pool material into the thalamic region of each hemisphere. Comparative evaluations shall be made with the virus pool under test and the Reference Attenuated Poliovirus, Type 1. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least 24 monkeys are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate virus pool material into the thalamus, additional monkeys may be inoculated in order to reestablish the minimum number of monkeys for the test.

(2) *Intraspinal inoculation.* Each of a group of at least five monkeys shall be injected intraspinally with 0.2 milliliter of virus pool material containing at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells, and each monkey in additional groups of at least five monkeys shall be injected intraspinally with 0.2 milliliter of a 1:1,000 and 1:10,000 dilution, respectively, of the same virus pool material. Comparative evaluations shall be made with the virus pool under test and the reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least four monkeys per group are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate intraspinally, additional animals may be inoculated in order to reestablish the minimum number of animals per group.

(3) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations by a qualified pathologist shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla, mesencephalon and motor cortex of each monkey in the groups injected with virus under test and those injected with the Reference Attenuated Poliovirus, Type 1, except that for animals dying during the test period, these examinations shall be made immediately after death. If at least 60

percent of the animals of a group survive 48 hours after inoculation, those animals which did not survive may be replaced by an equal number of animals tested as prescribed in paragraph (b) of this section. If less than 60 percent of the animals of a group survive 48 hours after inoculation, the test must be repeated. At the conclusion of the observation the animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristics of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus, Type 1, with respect to:

(i) The number of animals showing lesions characteristic of poliovirus infection;

(ii) The number of animals showing lesions other than those characteristic of poliovirus infection;

(iii) The severity of the lesions;

(iv) The degree of dissemination of the lesions; and

(v) The rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. These five factors may be weighted and interpreted as the Director, Center for Biologics Evaluation and Research, or the Director's delegatees deem appropriate. Among permissible interpretations, the factors may be considered in different ways for monkeys inoculated intraspinally and for monkeys inoculated intrathalamically. Other relevant factors in addition to those listed in paragraph (b)(3)(i) through (b)(3)(v) of this section, such as public health consequences, may be considered in evaluating neurovirulence test results. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrates that the neurovirulence of the test virus pool does not exceed that of the Reference Attenuated Poliovirus, Type 1.

(4) *Test with Reference Attenuated Poliovirus.* The Reference Attenuated Poliovirus, Type 1, shall be tested as prescribed in paragraphs (b)(1) and (b)(2) of this section at least once for every 10 production lots of vaccine, except that the interval between the test of the reference and the test of any lot of vaccine shall not be greater than 3 months. The test procedure shall be considered acceptable only if lesions of poliomyelitis are seen in monkeys inoculated with the reference material at a frequency statistically compatible with all previous tests with this preparation.

§ 630.18 Additional tests for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent, except for unavoidable bacteriophage and the intended attenuated live poliovirus. The vaccine shall be tested for the absence of other infectious agents, including polioviruses of other types or strains. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 milliliters of each monovalent virus pool shall be tested by inoculation into at least 10 healthy rabbits, each weighing 1,500 to 2,500 grams. Each rabbit shall be injected with a total of 1.0 milliliter intradermally in multiple sites, and subcutaneously with 9.0 milliliters, of the monovalent virus pool and the animals observed for at least 3 weeks. Each rabbit that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and the brain and organs removed and examined. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of cercopithecid herpesvirus 1 or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice, each weighing 15 to 20 grams, shall be inoculated intraperitoneally with 0.5 milliliter and intracerebrally with 0.03 milliliter of each monovalent virus pool. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and